

**WHAT IS CLAIMED:**

1                   1.       An expression plasmid comprising an RNA polymerase I (pol I) promoter  
2       and pol I terminator sequences, which are inserted between an RNA polymerase II (pol II)  
3       promoter and a polyadenylation signal.

1                   2.       The expression plasmid of claim 1 wherein the pol I promoter is proximal  
2       to the polyadenylation signal and the pol I terminator sequence is proximal to the pol II promoter.

1                   3.       The expression plasmid of claim 1 wherein the pol I promoter is proximal  
2       to the pol II promoter and the pol I terminator sequence is proximal to the polyadenylation signal.

1                   4.       The expression plasmid of claim 1 wherein the plasmid corresponds to a  
2       plasmid having a map selected from the group consisting of pHW2000, pHW11 and pHW12.

1                   5.       The expression plasmid of claim 1, further comprising a negative strand  
2       RNA virus viral gene segment inserted between the pol I promoter and the termination signal.

1                   6.       The expression plasmid of claim 5, wherein the negative strand RNA virus  
2       is a member of the *Orthomyxoviridae* virus family.

1                   7.       The expression plasmid of claim 6, wherein the virus is an influenza A  
2       virus.

1                   8.     The expression plasmid of claim 7, wherein the viral gene segment encodes  
2     a gene selected from the group consisting of a viral polymerase complex protein, M protein, and  
3     NS protein; wherein the genes are derived from a strain well adapted to grow in cell culture or  
4     from an attenuated strain, or both.

1                   9.     The expression plasmid of claim 6, wherein the virus is an influenza B  
2     virus.

1                   10.    The expression plasmid of claim 8 wherein the plasmid has a map selected  
2     from the group consisting of pHW241-PB2, pHW242-PB1, pHW243-PA, pHW245-NP,  
3     pHW247-M, and pHW248-NS.

1                   11.    The expression plasmid of claim 8 wherein the plasmid has a map selected  
2     from the group consisting of pHW181-PB2, pHW182-PB1, pHW183-PA, pHW185-NP,  
3     pHW187-M, and pHW188-NS.

1                   12.    The expression plasmid of claim 7, wherein the viral gene segment encodes  
2     a gene selected from the group consisting of an influenza hemagglutinin (HA) gene and a  
3     neuraminidase (NA) gene.

1                   13.    The expression plasmid of claim 12, wherein the influenza gene is from a

1 pathogenic influenza virus strain.

1 14. The expression plasmid of claim 12, wherein the plasmid has a map  
2 selected from the group consisting of pHW244-HA, pHW246-NA, pHW184-HA, and pHW186-  
3 NA.

1 15. A minimum plasmid-based system for the generation of infectious negative  
2 strand RNA viruses from cloned viral cDNA comprising a set of plasmids wherein each plasmid  
3 comprises one autonomous viral genomic segment, and wherein the viral cDNA corresponding to  
4 the autonomous viral genomic segment is inserted between an RNA polymerase I (pol I) promoter  
5 and terminator sequences, thereby resulting in expression of vRNA, which are in turn inserted  
6 between a RNA polymerase II (pol II) promoter and a polyadenylation signal, thereby resulting in  
7 expression of viral mRNA.

1 16. The minimum plasmid-based system of claim 15 wherein the pol I  
2 promoter is proximal to the polyadenylation signal and the pol I terminator sequence is proximal  
3 to the pol II promoter.

1 17. The minimum plasmid-based system of claim 15 wherein the pol I  
2 promoter is proximal to the pol II promoter and the pol I terminator sequence is proximal to the  
3 polyadenylation signal.

1                   18.     The plasmid-based system of claim 15, wherein the negative strand RNA  
2 virus is a member of the *Orthomyxoviridae* virus family.

1                   19.     The plasmid-based system of claim 18, wherein the virus is an influenza A  
2 virus.

1                   20.     The plasmid-based system of claim 18, wherein the virus is an influenza B  
2 virus.

1                   21.     The plasmid-based system of claim 19, wherein the viral gene segment  
2 encodes a protein selected from the group consisting of a viral polymerase complex protein, an M  
3 protein and an NS protein; wherein said genes are from a strain well adapted to grow in cell  
4 culture or from an attenuated strain, or both.

1                   22.     The plasmid-based system of claim 19, wherein the viral genomic segments  
2 comprise genes which encode a protein selected from the group consisting of hemagglutinin and  
3 neuraminidase, or both; wherein said genes are from a pathogenic influenza virus.

1                   23.     The plasmid-based system of claim 19 wherein said system comprises one  
2 or more plasmids having a map selected from the group consisting of pHW241-PB2, pHW242-  
3 PB1, pHW243 -PA, pHW244-HA, pHW245-NP, pHW246-NA, pHW247-M, and pHW248-NS.

1           24.     The plasmid-based system of claim 19, wherein said system comprises one  
2     or more plasmids having a map selected from the group consisting of pHW181-PB2, pHW182-  
3     PB1, pHW183 -PA, pHW184-HA, pHW185-NP, pHW186-NA, pHW187-M, and pHW188-NS.

1           25.     A host cell comprising the plasmid-based system of claim 15.

1           26.     A host cell comprising the plasmid-based system of claim 18.

1           27.     A host cell comprising the plasmid-based system of claim 19.

1           28.     A host cell comprising the plasmid-based system of claim 22.

1           29.     A method for producing a negative strand RNA virus virion, which method  
2     comprises culturing the host cell of claim 25 under conditions that permit production of viral  
3     proteins and vRNA or cRNA.

1           30.     A method for producing an *Orthomyxoviridae* virion, which method  
2     comprises culturing the host cell of claim 26 under conditions that permit production of viral  
3     proteins and vRNA or cRNA.

1           31.     A method for producing an influenza virion, which method comprises  
2     culturing the host cell of claim 27 under conditions that permit production of viral proteins and

1 vRNA or cRNA.

1 32. A method for producing a pathogenic influenza virion, which method  
2 comprises culturing the host cell of claim 28 under conditions that permit production of viral  
3 proteins and vRNA or cRNA.

1 33. A method for preparing a negative strand RNA virus-specific vaccine,  
2 which method comprises purifying a virion produced by the method of claim 29.

1 34. The method according to claim 33, which further comprises inactivating the  
2 virion.

1 35. The method according to claim 33, wherein the negative strand RNA virus  
2 is an attenuated virus.

1 36. A method for vaccinating a subject against a negative strand RNA virus  
2 infection, which method comprises administering a protective dose of a vaccine of claim 33 to the  
3 subject.

1 37. A method for vaccinating a subject against a negative strand RNA virus  
2 infection, which method comprises injecting a protective dose of a vaccine of claim 33  
3 intramuscularly in the subject.

1                   38.     A method for vaccinating a subject against a negative strand RNA virus  
2     infection, which method comprises administering a vaccine of claim 33 intranasally to the subject.

1                   39.     A method for generating an attenuated negative strand RNA virus, which  
2     method comprises:

- 3                   (a)     mutating one or more viral genes in the plasmid-based system of claim 15;  
4                   and  
5                   (b)     determining whether infectious RNA viruses produced by the system are  
6                   attenuated.

1                   40.     A composition comprising a negative strand RNA virus virion, wherein  
2     viral internal proteins of the virion are from a virus strain well adapted to grow in culture or from  
3     an attenuated strain, or both and viral antigen proteins, of the virion are from a pathogenic virus  
4     strain.

1                   41.     A composition comprising a negative strand RNA virus virion produced by  
2     the method of claim 29.